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Research Article

THE *IN VITRO* EFFECT OF DIFFERENT EXTRACTS OF *OCIMUM GRATISSIMUM* ON CUMEN HYDROPEROXIDE–INDUCED OXIDATIVE STRESS IN RAT LIVER TISSUE

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ABSTRACT
Extracts of *Ocimum gratissimum* (EOG) were prepared using solvents of increasing polarity in succession: hexane (HEOG), dichloromethane (DEOG), butanol (DEOG), methanol (MEOG) and the aqueous extract (AEOG). The antioxidant potential of the extracts was assessed by determining their total phenolic content and DPPH radical scavenging ability. The radical scavenging activity of the extracts generally increased with polarity of the solvent and was positively correlated with the total phenolic content. The extracts were then fed to rats with cumen hydroperoxide-induced oxidative stress. Subsequent *in vitro* assays conducted on the rat liver homogenates (toxicity studies, estimation of lipid peroxidation, estimation of reduced glutathione (GSH), estimation of superoxide dismutase (SOD), estimation of catalase (CAT) and estimation of protein), showed that the plant extracts protected the liver tissue of the experimental group, prevented oxidative stress and were not toxic to the rats. The overall performance of the extracts in protecting the liver was found to be in the same order as their total phenolic content and radical scavenging ability: MEOG > BEOG > DEOG > HEOG > AEOG. The aqueous extract was the least active because it was the last solvent used in the extraction cycle after the previous solvents had extracted most of the phenolic compounds responsible for the antioxidant and therapeutic capacity of the extracts.

Key Words: *Antioxidants, Reactive Oxygen Species, Oxidants, Oxidative Stress, Lipid Peroxidation*

INTRODUCTION
Liver is the largest internal organ of the human body which metabolizes a variety of foreign substances, some of which are converted to metabolic products thus playing a major role in detoxification processes. Histologically the liver tissue is formed mainly of hepatocytes which are the functional cells and perform a wide variety of metabolic, secretory and endocrine functions (Chauhan *et al.,* 1992). A free radical is defined as any atom or molecule that possesses an unpaired electron and it can be anionic, cationic or neutral. In biological and related fields, the major free radical species of interest have been those of oxygen that is oxygen free radicals (OFRS). The term OFRS includes the superoxide anion free radical (O_2^{-*}), the hydroxyl radical ($HO^{\cdot}$) lipid radical (L) peroxy radicals ($LOO^{\cdot}$ and $XOO^{\cdot}$) (Finkel and Holbrook, 2000) and more recently through research into nitrous oxide radical ($NO^{\cdot}$). OFRS are potentially very toxic to cells due to their reactive nature. They can readily combine with other molecules such as enzymes, receptors and ion pumps causing oxidation directly and inactivating or inhibiting their normal functions. Some of the products of OFRS attack other molecules and can interfere with nucleic acid function hence causing mutations leading in extreme pathological situations to cancers or germ line mutations (Gulcin, 2010). Oxidants are formed as a normal product of aerobic metabolism but can be produced at elevated rates under pathophysiological conditions. In cell metabolism, oxidants may exist and may be transported to distant target sites where they exert oxidant activity. An antioxidant can be defined as any substance that when present at low concentrations significantly delays or inhibits the activity of the oxidants and prevent the formation of free radicals and hence OFRS. An imbalance

between oxidants and antioxidants infavour of the oxidants, potentially leading to damage, is termed 'oxidative stress' (Astiz *et al.*, 2009).

Medicinal plants have been used as therapeutic agents for centuries because of the presence of secondary metabolites, referred to as 'phytochemicals', that have been shown to be effective in combating or preventing disease due to their antioxidant ability (Anita *et al.,* 2010). *Ocimum* species are known for their medicinal properties and are used extensively in Ayurvedic preparations (Geetha and Vasudavan, 2004). *O. gratissimum* commonly known as wild basil also belongs to Lamiaceae family, highly branched shrub of about 1-2 meters height, with ribbed stem and laborious branches. Its leaves are simple, oppositely arranged, 3-5 centimeters long, 1.2 centimeters wide (Akinmoladun *et al.,* 2007). The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Afolabi *et al.,* 2007).

The present work has been designed to evaluate the antioxidant potential of different extracts of *O. gratissimum* and its *invitro* effect on cumen hydroperoxide induced oxidative stress on hepatic tissue in liver homogenate.

MATERIALS AND METHODS

Collection and Identification of Plants

Tender parts of *O. gratissimum* were collected locally from Botswana and the identification of the plant was done by Dr. M. P. Setshogo at the University of Botswana Herbarium (UCBA). The voucher specimen was submitted in the herbarium and voucher No. was given as (2006/G, A01).

Preparation of the Extracts

Plants were collected locally, washed with distilled water and sun dried. The dried plant materials were crushed with a blender or laboratory grinder to obtain a coarse powder. About 750 grams of the above powder was soaked and extracted successively at room temperature in the following solvent system: 100% hexane followed by butanol, dichloromethane, methanol and the distilled water. The extracts were filtered and made solvent-free by evaporating in a Buchi-type rotary evaporator at 65^0C. They were labeled according to the solvents used as, HEOG,BEOG DEOG,MEOG,AEOG and the yield were 7.8% , 7.2, 6.4, 8.2 and 4.2(W/W) respectively.

Animal's Male albino rats of Wister strain of body weight ranging 200-250 grams were housed in colony cages at ambient temperatures of $25 \pm 2^{\circ}$C and 50-55% relative humidity with 12 hours light and dark cycle. They had water and food *ad libitum.* Experiment was conducted as per the internationally accepted principles for laboratory animal care unit of University of Botswana.

Chemicals

All the chemicals used were analytical grade and bought from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA. Reagents DPPH (2,2-diphenyl-l-picrylhydrazyl) reagent [molecular formula C18H12N5O6 molecular weight 394 g/mol] was purchased from Fluka Chemicals (Steinheim, Germany), and Folin-Ciocalteau reagent from Rochelle Chemicals (South Africa) Ascorbic acid and anhydrous sodium carbonate were all analytically pure and were purchased from Unilab (South Africa). Gallic acid (AR) was obtained from Sigma Chemicals (Steinheim, Germany). The solvents used for the extraction process were also of analytical grade obtained from Clover chemicals Botswana. The TLC sheets were ready made, aluminium backed and coated to a thickness of 0.25 mm with silica gel 60 F254brought from Rochelle Chemicals (South Africa).

Radical Scavenging Activity

DPPH Spectrophotometric Method

The free radical scavenging activity was measured using the DPPH method modified by Yeboah and Majinda (2009). A solution of 500 µM DPPH (i.e. 0.02 % or 0.2 mg/mL) in methanol (AR) was prepared. Also different concentrations of each of the plant extracts and standards, ascorbic acid (AA) and gallic acid (GA), were prepared ranging from 0.05-0.5 mg/mL in methanol. Each extract or standard solution (2

mL) was added to an equal volume of the DPPH solution, making a total reaction volume of 4 mL. A control reaction mixture was prepared consisting of 2 mL methanol without extract and an equal volume of DPPH solution. The test tubes were tightly closed, vigorously shaken and placed in a dark cupboard for 30 minutes. The absorbance of each solution was measured at 517 nm after 2 hours and finally after 24 hours. Methanol was used as the blank for baseline correction. The percentage inhibition of DPPH, I% was calculated using the following formula:

$$I\% = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \text{ x } 100}{\text{Absorbance of control}}$$

From the inhibition curves (I% versus sample concentration in µg/mL) the concentration of extract or standard required to inhibit DPPH radical activity by 50% (IC_{50}) was determined using non-linear regression equations that best fitted the curves. The experiment was carried out in triplicate and the IC_{50} values (µg/mL) were reported as the average of three trails ± the standard deviation.

Total Phenolic Content

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu reagent method as described by Yeboah and Majinda (2009). Five different concentrations of the standard, gallic acid, in methanol were prepared ranging from 0.01 to 0.05 mg/mL. 5 mL of 90% aqueous methanol and 0.5 mL Folin-Ciocalteu reagent were added to 0.5 mL of each of the standard solutions and to 0.5 mL of each extract solution (1 mg/mL) in screw cap test tubes. After 3 min, 1 mL of 2% Na_2CO_3 was then added to each test-tube and the mixture was vigorously shaken for 2 minutes and left to stand for 2 hours at room temperature.

The absorbance of the supernatant solution was determined at 725 nm using 90% aqueous methanol as a solvent blank. A gallic acid standard curve was prepared and the equation derived by linear regression (y =36.84 x+0.1069) was used to determine the TPC of each extract in mg of gallic acid equivalents/g of extract (mg GAE/g). The experiment was performed in triplicate and TPC was reported as the average value of 3 trials ± the standard deviation.

Preparation of the Liver Homogenate

Rats were dissected and the liver was perfused with phosphate buffer saline through the hepatic portal vein. The lobes of the liver were collected and dried between filter papers to remove the excess blood. It was then cut with a heavy duty blade into smaller pieces and transferred to a glass Teflon homogenizing tube to prepare the homogenate (1 gram of the liver tissue) in phosphate buffer saline (pH7.4) in 4 ^{0}C. Then the liver tissue was centrifuged at 2000 rpm for ten minutes and the supernatant was taken for further use.

Experimental Design

Effect of Plant Extracts on Liver Homogenate

This was conducted to establish that the extract had no toxic effect on liver homogenate and was safe to be used. Here the 10% liver homogenate was incubated with the extracts only for 60 minutes to find these extracts produce any positive or negative effects on liver tissue.

The groups were divided as (1) NC –normal control with normal diet and distilled water followed by the different extract treated groups as ET. Five replicates of liver homogenate were mixed with different extracts of the plant (300 µL of the 10% solution), which was incubated for 60 minutes. The biochemical estimations were done on TBARS, GSH, SOD and CAT after 60 minutes (George and Chaturvedi, 2013). Five replicates of the following groups were used-

1. NC- Normal control– 3mL of Liver homogenate +250µ L of Distilled Water
2. ET 1 Extract treated– 3mL Liver homogenate+ 250µ L of MEOG
3. ET 2 Extract treated – 3mL Liver homogenate+ 250µ L of DCEOG
4. ET 3 Extract treated – 3mL Liver homogenate+250µ L of BEOG
5. ET 4 Extract treated – 3mL Liver homogenate+ 250mL of HEOG
6. ET 5 Extract treated – 3mL Liver homogenate+ 250µ L of AEOG

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Effect of the Extracts on CHP- induced Lipid Peroxidation on Liver Homogenate

In this experiment, the antioxidant property of plant extracts were investigated in CHP induced lipid peroxidation. The groups were (1) normal control with normal diet and distilled water (2) CC- cumen hydroperoxide control (3) followed by extracts treated EX groups treated with different extracts. Five replicates of the following groups were used.

1. NC- 3 mL homogenate+1.3 mL of distilled water- normal control
2. CC- 3 mL homogenate +1mL CHP+ 300 µ L of distilled water.
3. EX1- 3 mL homogenate +1mL CHP +300 µ L of the HEOG
4. EX2- 3 mL homogenate +1mL CHP +300 µ L of the DEOG
5. EX 3- 3 mL homogenate +1 mL CHP +300 µ L of the BEOG
6. EX 4- 3 mL homogenate +1 mL CHP +300 µ L of the MEOG
7. EX 5- 3 mL homogenate +1 mL CHP +300 µ L of the AEOG

200 µ L of the above mixture was pipetted from each group at 0, 20, 40 and 60 minutes and estimated the quantity of TBARS, GSH, SOD and CAT were estimated in each of them.

Estimation of Lipid Peroxidation

TBARS in liver homogenate was estimated by the method described by Niehaus (1986) with few modifications. Lipid peroxidation was measured by the formation of thiobarbituric acid reactive substances (TBARS) such as malonyl dialdehyde (MDA). MDA formed from breakdown of fatty acids, served as a convenient index for determining the extent of peroxidation reaction. MDA has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give red species absorbing at 535 nm. 3 mL of liver homogenate was treated with 2 mL of 10% TCA-TBA-HCL (1:1:1) mixture and incubated in boiling water bath for ten minutes, then the mixture was cooled, to that added 2mL of freshly prepared 1N NaOH and the absorbance was measured at 535 nm.

Estimation of Reduced Glutathione (GSH)

Reduced glutathione was estimated by the method of Ellman (1959). 3 mL of liver homogenate was mixed with 0.5 mL of precipitating buffer (5% TCA in 1m M EDTA), centrifuged and supernatant was collected. This was mixed with 2.5 mL of 0.1 M phosphate buffer (pH 8.0). The colour was developed by adding 100 µl DTNB (0.01%) and the absorbance was measured at 412 nm.

Estimation of Superoxide Dismutase (SOD)

Superoxide Dismutase was assayed by the method of Kakkar *et al.,* (1984). The role of SOD is to accelerate the dis-mutation of the superoxide radical ($Oz^{\cdot}$) produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. The assay of SOD activity is based on the principle of inhibitory effects of SOD on reduction of nitro blue tetrazolium dye by superoxide radicals. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue tetrazolium) reduction /min/mg protein.

The reaction mixture contained 150 µl EDTA, 600 µl L-methionine, 300µl NBT and the volume was made up to 2.8 mL by the addition of SOD buffer. To the reaction mixture was added 3 µl of the liver homogenate except in the control. Finally to this was added 200 µl of riboflavin to start the reaction. The test tube was kept under a fluorescent lamp and the reaction kinetics measured. The absorbance was taken at 560 nm for 60 minutes.

Estimation of Catalase (CAT)

Catalase was estimated by the method of Hans Bisswagner (2004). To 0.98 mL of H_2O_2 – solution (10 mM), 0.2mL of liver homogenate was added. The absorbance was taken at 240 nm and the catalase activity was calculated using the extinction coefficient of H_2O_2 – (0.071). The activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

Estimation of Protein

Protein was determined by the method of Lowry *et al.,* (1951) using Bovine Serum albumin as standard, at 660 nm. One ml of sample was mixed with 3 ml of Lowry's reagent (prepared by mixing 98ml of 2% sodium bicarbonate solution, 1 ml of 1% copper sulfate solution, and 1 ml of sodium potassium tartrate) and incubated for 10 minutes at room temperature. After incubation, 0.3 ml of Folin-Ciocalteau reagent

(diluted with equal volume of water) was added. The color formed was read against blank (1 ml of distilled water) at 670 nm after 30 minutes

Statistical Analysis

All data are expressed as the mean ±S.E. mean of n=6. Analysis of variance was performed by one-way ANOVA and the significant differences between the means were determined by Holm-Sidak method at *p*-value of ≤0.05. Statistical software Sigmastat 3.1 was used to analyze the data.

RESULTS AND DISCUSSION

Total Phenolic Content of Different Extracts of Ocimum gratissimum

The TPCs determined for the extracts are given in Table (1). The results show that, apart from the aqueous extract (AEOG), TPC generally increased with increase in polarity of the extracts; MEOG > DCEOG > BEOG > AEOG > HEOG. The aqueous extract had lower than expected TPC which could be attributed to the fact that it was the last solvent used in the sequence of extractions.

Table 1: Total phenolic content of different extracts of *Ocimum gratissimum*

Extracts	TPC (mgGAE/g)*
MEOG	254.21± 0.43
DCEOG	134.02 ±0 .04
BEOG	65.45 ± 1.29
HEOG	44.87 ±0 .56
AEOG	63.17 ±1.21

**Values are the average of three trials ± standard deviation*

Radical Scavenging Activity

DPPH Spectrophotometric Method

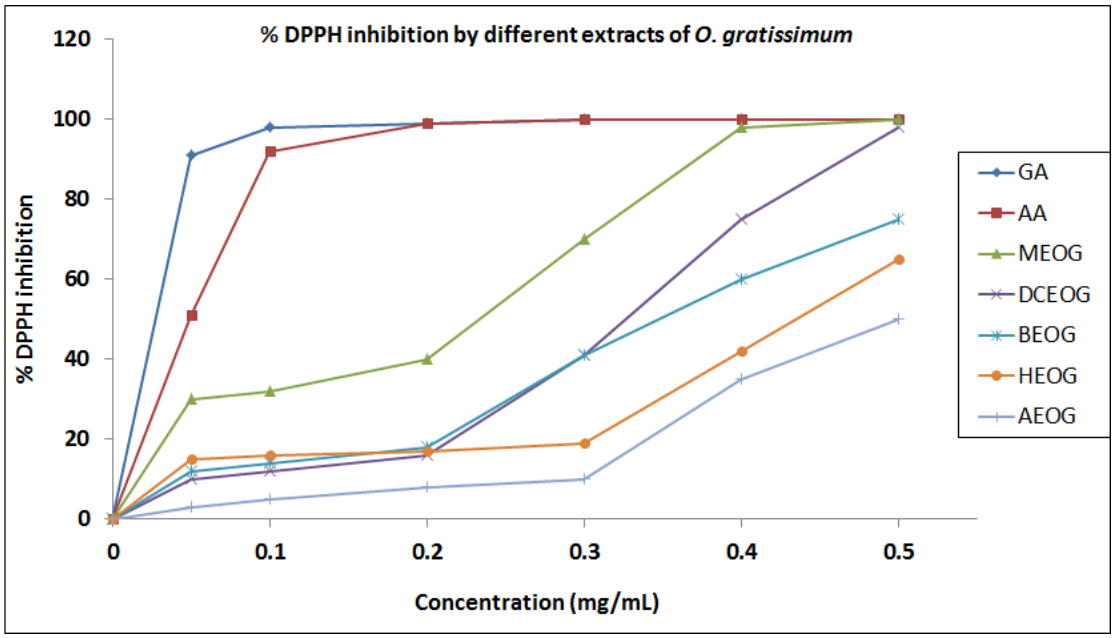

Figure 1: DPPH activity of extracts of *Ocimum gratissimum*

The radical scavenging power (RSP) of the extracts in comparison with the standards was found to be in the order: GA > AA > MEOG > DCEOG > BEOG > HEOG > AEOG (Figure 1) and this was positively correlated with TPC (r^2 = 0.836) suggesting that phenolic compounds are probably responsible for the radical scavenging activity displayed by the extracts of *O. gratisssimum*. Many *Ocimum* species have indeed been found to possess phenolic compounds such as euginol, methylchavicol, linalool in their chemical composition (Prabhu, 2009). The strong RSP displayed by the standards, AA and GA was comparable to that reported by Thabrew *et al.,* (1998).

Effect of the Extracts on Liver Homogenate

The results of the effect of the extracts on liver homogenate (Table 2) indicate the fact that the extracts used did not change the antioxidant parameters checked significantly but there were slight elevation of the enzymatic antioxidants such as CAT and SOD and non-enzymatic antioxidant such as GSH when compared with the normal control. TBARS levels were not changed significantly when compared with the NC group but a slight decrease was shown in ET-1 and ET-2. From the result obtained it was clear that these extracts did not have any toxic effect and can be used for *in vitro* studies without any toxic effect of its own.

Table 2: Effect of the extracts on liver homogenate after 60 min

S NO	GROUPS	TBARS (n mol/g wet tissue)	GSH (mg /g /wet tissue)	CAT (Umg/-^{1} protein	SOD (Umg/-^{1} protein
1	**NC**	2.56 ± .06	31.83± .05	71.73± .06	3.23± .03
2	**ET1**	2.53 ± .02	40.76 ± .64	80.46 ± .06	3.61 ± .04
3	**ET2**	2. 53 ± .04	38.45 ±.55	78.65 ±.09	3.41 ±.08
4	**ET3**	2.55 ±.03	36.88 ±.12	76.88 ±.45	3.39 ±.06
5	**ET4**	2.56 ±1.21	32.51 ±.51	74.32 ±.09	3.28 ±.1
6	**ET5**	2.56 ± .13	31.89 ±.22	71.87 ±.03	3.24 ±.06

n=5 replications in each group, p<0.001

1. NC- Normal control– 3mL of Liver homogenate +250µ L of Distilled Water
2. ET 1 Extract treated– 3mL Liver homogenate+ 250µ L of MEOG
3. ET 2 Extract treated – 3mL Liver homogenate+ 250µ L of DCEOG
4. ET 3 Extract treated – 3mL Liver homogenate+250µ L of BEOG
5. ET 4 Extract treated – 3mL Liver homogenate+ 250mL of HEOG
6. ET 5 Extract treated – 3mL Liver homogenate+ 250µ L of AEOG

The effects of the different extracts on liver homogenate was checked to find the toxic effect of the extract on liver tissue and it was established that the extract was safe to be used without any toxic effects. All the parameters checked after 60 minutes (Table 2) did not show any significant difference from the normal control used. This was in agreement with results obtained in phytochemical analysis and antioxidant activity of *O.gratissimum* extracts from leaves by Acinomoldanin (2007).

The in vitro Effect of the Extracts on CHP- induced Lipid Peroxidation

The present study was conducted to evaluate the protective effect of different extracts on liver tissue in liver homogenate. The lipid peroxidation was induced by cumene hydroperoxide because other toxicants

need much higher doses to induce the lipid peroxidation in a shorter time. This was in agreement with the findings of Geenley and Davis (1992) to use azo initiators such as cumene hydroperoxide in which the alkoxyl radicals can undergo **β** –scission to give methyl radicals. The results suggest that the extract possess protective action against hepatic dysfunctions induced by CHP in dose and time dependent manner. The optimum dose was established as 300 μ L of the extracts in 3mL of liver homogenate by the same authors (George and Chaturvedi, 2013). The results indicate a strong evidence of preventing the lipid peroxidation and protecting the liver tissue from the toxicity induced by cumene hydroperoxide.

Table 3: Effect of the extracts on TBARS in CHP-induced lipid peroxidation in liver homogenate

SNO	GROUPS	0-MIN (n mol/g wet tissue)	20-MIN (n mol/g wet tissue)	40-MIN (n mol/g wet tissue)	60-MIN (n mol/g wet tissue)
1	**NC**	2.64 ± .09	2.65 ± .03	2.66 ± .04	2.69 ± .18
2	**CC**	2.95 ± .28	6.41 ± .15*	7.59 ± .07*	7.62± .03*
3	**EX 1**	2.55 ± .43	1.61 ± .06	1.92 ±.05	2.51 ± .13
4	**EX-2**	2.55 ± .72	1.77 ±.09	1.98 ±.04	2.54 ±.08
5	**EX-3**	2.55 ± .9	1.99 ±.05	2.09 ±.06	2.61 ±.09
6	**EX-4**	2.57 ±.04	2.08 ±.21	2.11 ±.07	2.64 ±.05
7	**EX-5**	2.57.09	2.43 ±.81	2.54 ±.11	2.66 ±.09

Table 3 shows the *in vitro* effects of different extracts on TBARS checked in 60 minutes. The trend showed that the CHP treated group CC showed the lipid peroxidation within 20 minutes time, up to 60 minutes and it was increasing with time. When we look at the experimental groups which were treated with the extract together with CHP, the amount of TBARS produced was normal almost comparable with the control group. The levels of TBARS were increased in CC groups which clearly indicated the amount of malondialdehyde (MDA). MDA was identified as the product of lipid peroxidation by Dargel (1992).

Table 4: Effect of the extracts on GSH in CHP-induced lipid peroxidation in liver homogenate

SNO	GROUPS	0-MIN (mg /g /wet tissue)	20-MIN (mg /g /wet tissue)	40-MIN (mg /g /wet tissue)	60-MIN (mg /g /wet tissue)
1	**NC**	31.96 ± .06	31.66 ± .04	32.46 ± .04	32.99 ± .04
2	**CC**	30.98 ± .14	16.41 ± .16*	21.59 ± .08*	33.86 ± .07
3	**EX 1**	31.96 ± .05	42.61 ± .06	43.02 ±.05	44.98 ± .13
4	**EX-2**	31.88 ± .96	41.85 ±.09	42.44 ±.08	43.74 ±.32
5	**EX-3**	31.66 ±65	40.44 ±.09	40.88 ±.04	42.65 ±. 64
6	**EX-4**	31.23±.08	39.88±.4	39.96±.12	40.21±.43
7	**EX-5**	31.21±.06	36.73±.09	37.66±.07	38.23±.05

The decrease in GSH levels in CC groups after 20 minutes indicates the toxicity induced by the presence of CHP. This was due to the decreased availability of GSH caused by enhanced lipid peroxidation. In the present study, a significant decrease in the GSH content in the CC group as compared to all the experimental groups and the NC group was also observed. GSH inhibits the generation of reactive oxygen species and hence the oxidative stress that damages the structural integrity of cell membrane and membrane organelles.

Table 5: Effect of the extracts on CAT in CHP-induced lipid peroxidation in liver homogenate

SNO	GROUPS	0-MIN (Umg/-^{1} Protein)	20-MIN (Umg/-^{1} Protein)	40-MIN (Umg/-^{1} Protein)	60-MIN (Umg/-^{1} Protein)
1	**NC**	71.56 ± .12	71.66 ± .08	71.46 ± .04	71.39 ± .03
2	**CC**	70.61± .02	36.41 ± .15*	41.59 ± .07*	63.86 ± .03*
3	**EX 1**	72.12 ± .58	72.61 ± .04	73.05 ±.09	80.68 ± .17
4	**EX-2**	71.86 ±.09	72.43 ± .03	72.88 ± .03	78.93 ± .8
5	**EX-3**	71.43 ±.07	72.21 ±.11	72.56 ± .04	77.86 ±.09
6	**EX-4**	71.31 ±.41	71.89 ± .09	71.85 ± .03	76.83 ±.8
7	**EX-5**	71.11 ±.09	71.55 ±.88	71.63 ±.05	75.43 ± .04

Table 6: Effect of the extracts on SOD in CHP-induced lipid peroxidation in liver homogenate

SNO	GROUPS	0-MIN (Umg/-^{1} Protein)	20-MIN (Umg/-^{1} Protein)	40-MIN (Umg/-^{1} Protein)	60-MIN (Umg/-^{1} Protein)
1	**NC**	3.65 ± .18	3.66 ± .06	3.46 ± .12	3.99 ± .17
2	**CC**	3.63 ± .36	1.41 ± .15*	1.59 ± .05*	2.86 ± .07*
3	**EX 1**	3.64 ± .03	2.61 ± .03	3.02 ± .06	3.98 ± .17
4	**EX-2**	3.64 ± .09	2.54 ±.08	3.01 ±.09	3.79 ±.03
5	**EX-3**	3.62 ±.07	2.32 ± .71	2.98 ± .03	3.87 ±.05
6	**EX-4**	3.61 ±	2.30 ± .06	2.86 ± .12	3.69 ±.09
7	**EX-5**	3.61 ±	2.28 ± .04	2.67 ± .11	3.88 ±.04

**Considered as significantly different*
n=5 replications in each group, p<0.001

1. NC- 3 mL homogenate+1.3 mL of distilled water- normal control
2. CC- 3 mL homogenate +1mL CHP+ 300 μ L of distilled water
3. EX1-3 mL homogenate +1mL CHP +300 μ L of the HEOG

4. EX2- 3 mL homogenate +1mL CHP +300 μ L of the DEOG
5. EX3- 3 mL homogenate +1 mL CHP +300 μ L of the BEOG
6. EX4- 3 mL homogenate +1 mL CHP +300 μ L of the MEOG
7. EX5- 3 mL homogenate +1 mL CHP +300 μ L of the AEOG

In all the parameters checked, there were significant difference between the NC groups and CC groups but not significantly different from the EX groups, which showed that the extract could prevent the lipid peroxidation and the depletion of its indigenous antioxidants by scavenging the free radicals (George and Chaturvedi, 2009). TBARS are one of the diagnostic indices of lipid peroxidation due to oxidative stress. Thus, in the present study, high levels of TBARS in the CC group indicate a high rate of lipid peroxidation, which ultimately causes membrane damage and cellular death (Table 3). Levels of TBARS are significantly low in all experimental groups, indicating inhibition of lipid peroxidation by the extracts used. It has been reported that SOD, CAT and GSH constitute a mutually supportive team of defense against ROS (Awah *et al.,* 2010). ROS easily react in vitro with most biological molecules, causing their degradation and destruction. Evidence suggests that various enzymatic and non-enzymatic have been developed by liver cells to cope with the production of ROS and free radicals. The decrease in GSH has been related to an enhanced oxidation of GSH to oxidized glutathione (GSSG) as a consequence of increased generation of reactive oxygen species (Anitha *et al.,* 2011). As such, it is an important antioxidant defense in nearly all cells exposed to oxygen. Hydrogen peroxide is then converted to oxygen and water by glutathione peroxidase and catalase and they constitute a mutually supportive team of defense against ROS (Gulcin *et al.,* 2007). SOD is fundamental in the processing of reactive oxygen species by reducing superoxide anion to form hydrogen peroxide. Catalase and glutathione peroxidase further reduce hydrogen peroxide to H2O. Increased hydrogen peroxide slowly inactivates CuZn- SOD. Therefore, catalase and glutathione peroxidase, by reducing hydrogen peroxide, conserve SOD; and SOD, by reducing superoxide, in turn conserves catalase and glutathione peroxidase. A steady low level state of SOD, glutathione peroxidase, and catalase, as well as superoxide and hydrogen peroxide are thus maintained by such a feedback mechanism.

In conclusion, the results of the present study reveal that the treatment with these extracts could prevent the lipid peroxidation induced by cumene hydroperoxide and renders protection against CHP-induced toxicity in rat liver tissue. Moreover, these extracts are safe to be used without any hepatic toxicity, and the protective mechanisms of these extracts seem to be increasing with polarity of the solvents used.

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